Cultivability and survey of soil mycoflora and microflora from vermicompost and undisturbed sample

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Hypothesis:

- By the use of contemporary and conventional isolation techniques of microflora and mycoflora, vermicompost will have a greater biodiversity than a undisturbed sample.

Introduction:

Compost has been proven to be an alternative strategy in maintaining healthy arable regions. Due to the increased biological activity within compost, driven by three broad groups of microorganisms: psychrophiles, mesophiles and thermophiles, various composting methods can degrade even complex polymers and plastics. Vermicompost, a product by the presence of earthworms, contains a greater number of fungal entities and biodiversity in contrast to thermophically produced green compost (Anastai et al 2005). An undisturbed sample collected from a deciduous forest should have a lower species diversity than either vermicompost or green compost. Earthworms stabilize organic residues and reduce pathogenic bacteria (Eastman et al 2001). Through the selection of spore germination and creation of unique microsites, earthworms are able to influence fungal growth that could provide favorable or unfavorable conditions to specific species (Brown 1995, Tiunov and Scheu 2000). From industrial-scale bioremediation of waste management to small-scale gardening, compost has vast applications.

It has been estimated that 1% of microorganisms have been isolated from soil using conventional extraction techniques (Davis et. al 2005). The biomass ratio of fungi and prokaryotes in compost is about 2:1 (Sparling et al 1982, Wigen 1992). Isolation and cultivation of fungal species from soil is limited using conventional techniques in a laboratory setting. Utilization of exudate from fungi has been applied from large-scale industrial projects that focus on improving water quality to the production of β-lactam antibiotics. Therefore, new techniques in media preparation and isolation techniques are necessary in order to fully take advantage of potential new biotechnology.

Materials:

Growth Media:

- Sabouraud Dextrose Agar (SDA)
- Potato Dextrose Agar (PDA)
- PDA with Nystatin (PDAn)
- Mannitol Salt Agar (MSA)
- MacConkey Agar (MCA)
- Eosin Methylene Blue Agar (ESB)
- Tryptic soy agar (TSA)
- TSA with Nystatin (TSAn)

Stain:

- Lactophenol Cotton Blue (LPCB)

Methods:

Soil Dilution:

•Soil was diluted to 1:1,000 g/mL with sterile water. For vermicompost and undisturbed sample, 1mL was pipetted out of dilution and centrifuged for 5 minutes. This was performed in triplicate for each media. Isolates were grown on slide cultures and then stained with LPCB to identify reproductive structures.

Fig. 1: Undisturbed Sample. Note the light brown color and particulate size.

Fig. 2: Vermicompost Sample. Note the dark pigmentation and particulate size.

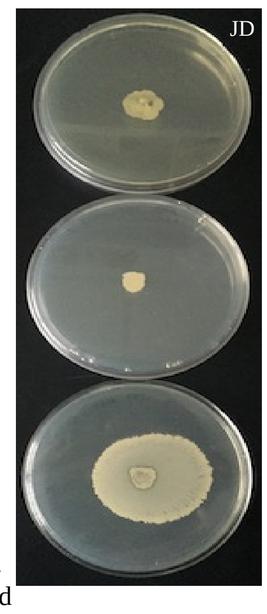

Fig. 3: From top to bottom: bacteria cultured on SDA, TSAn and TSA. Note numerous radial projections on TSA culture. Radial projections were completely absent when grown on SDA and TSAn.

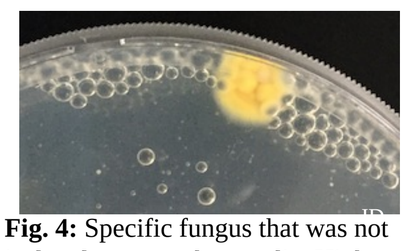

Fig. 4: Specific fungus that was not isolated on any other media. High frequency of bubbles are meant to replicate 'microsites' created by earthworms. Soil particles are larger in vermicompost than in undisturbed sample. Electrostatic attraction holds the soil particles together creating small 'oxygen pockets' within the compost that create unfavorable and favorable conditions for microorganisms.

Macromorphology:

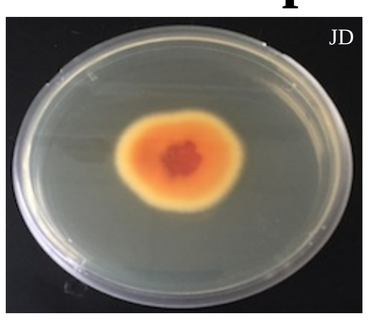

Fig. 5: Unknown fungus on SDA (bottom).

Fig. 6: *Bacillus mycoides* on SDA (top).

Fig. 7: *Penicillium* spp. on SDA (top).

Fungal Asexual Reproductive Structures:

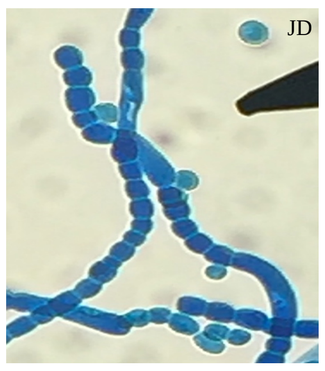

Fig. 8: Blastocondia (10x).

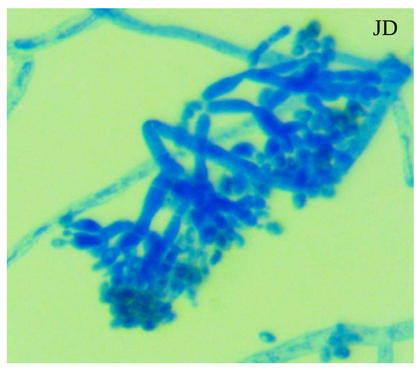

Fig. 9: Blastospores (40x).

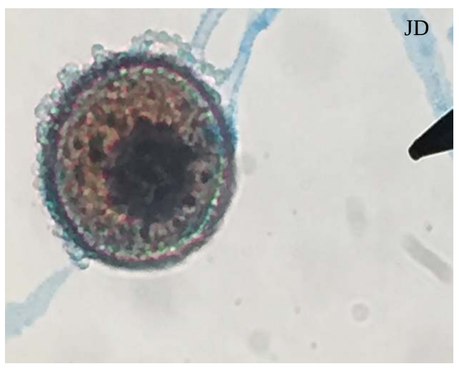

Fig. 10: Sporangium (40x).

Results:

Table 1: Number of fungal and bacterial morphological species isolated from vermicompost (VC) and undisturbed sample (UD). Number of isolates from VC only and UD only are in brackets. Morphologically distinct fungal and bacterial colonies when grown in culture are referred to as isolates or morphological species.

Table 1:	Vermicompost		Undisturbed Sample	
	Fungal Isolates:	Bacterial Isolates:	Fungal Isolates:	Bacterial Isolates:
Total:	82 (64)	7 (2)	64 (46)	6 (1)
PDA 28 C:	31 (23)	-	45 (37)	-
SDA 28 C:	51 (41)	-	19 (9)	-
TSA 28 C:	-	7 (2)	-	6 (1)

Table 2: Oneway analysis of Number by Microorganisms.

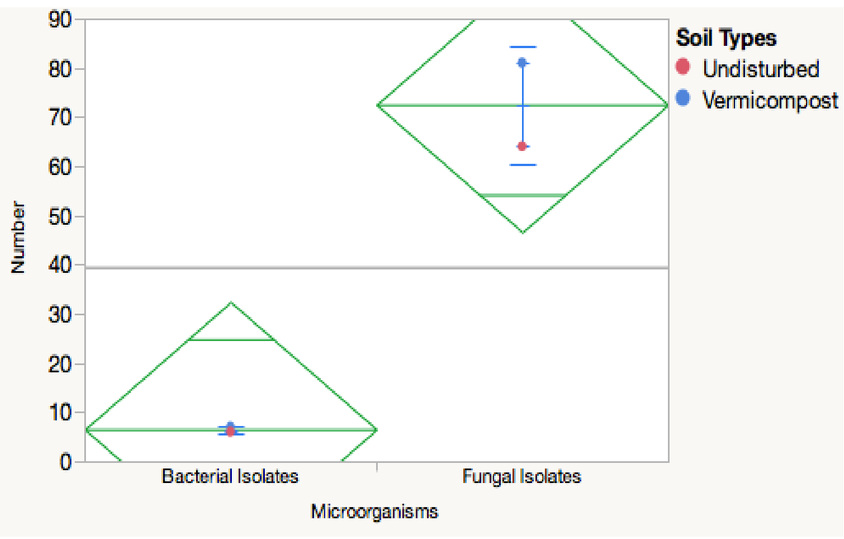

Table 2: Prob>F = 0.0153 showing significance. Illustrates the greater number of fungal morphological species isolated in comparison to bacterial species. This is not to say, there were more fungal species in each sample tested. In a laboratory setting most fungi have faster growth rates than bacteria. *Penicillium* for example typically sporualtes within 48 to 72 hours.

Table 3: Oneway analysis of Number of Isolates by Type of Media

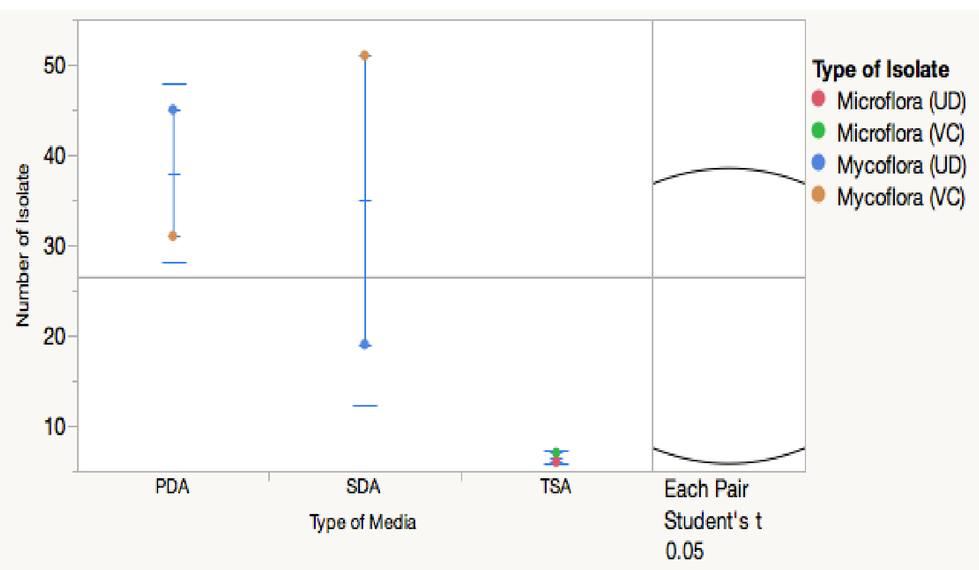

Table 3: Prob>F = 0.1944 showing insignificance. Demonstrates the effect of media type on the isolation of mycoflora/microflora. SDA and PDA being ineffective for the isolation of bacteria without fungicides.

Conclusion:

- Media composition can greatly influence the morphology and biochemistry of microorganisms.
- Different media preparation techniques greatly expands the range for the isolation of microorganisms that are either fastidious, slow-growing or requires a unique microsite for cultivation (see Fig. 4).
- Vermicompost possessed a higher number of fungal morphological species than undisturbed sample. However, the number of bacterial morphological species isolated was relatively the same.
- All isolations were performed using a 1:1,000 g/mL dilution. Illustrating the high frequency of species and variants present in such a small quantity.

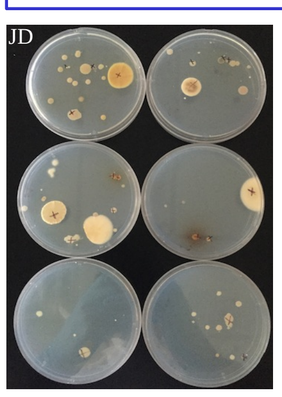

Fig. 11: One trial of isolation from UD on PDA. On six plates 19 morphologically distinct fungal species were counted.

References:

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- Davis KER, Joseph SJ, Jansen PH. 2005. Effect of growth medium, inoculum size and incubation on culturability and isolation of soil bacteria. Appl Environ Microbiol 71:826-834
- Eastman BR, Kane PN, Edwards CA, Trytek L, Gunadi B, Stermer AL, Mobley JR. 2001. The effectiveness of vermicolture in human pathogen reduction for USEPA biosolids stabilization. Compost Science & Utilization 9:38-49.

Acknowledgements

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